SD087
approved 4/98

Society of Environmental Toxicology and Chemistry
8th Annual Meeting of SETAC-Europe
14-18 April 1998, Bordeaux, France

# Use of a Rapid Bioluminescent Bioassay (QwikLite) Using Oceanic Dinoflagellates to Assess Toxicity in Sediments

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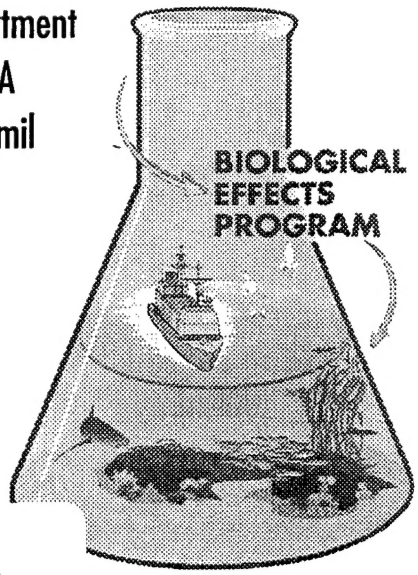

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# Use of a Rapid Bioluminescent Bioassay(QwikLite) Using Oceanic Dinoflagellates to Assess Toxicity in Sediments

C.H. Liu[2], D. Lapota[1], D.E. Rosenberger[2]

## Abstract

The Navy has developed a rapid bioassay system (QwikLite) that is proving to be a valuable asset for conducting bioassays on other test media (e.g., metals, storm drain discharge, ship hull coatings, and marine sediments). The basis of detection is to measure a reduction in light from the bioluminescent dinoflagellate *Gonyaulax polyedra* following exposure to a toxicant. The toxic response is usually measured within 24 hours from the start of the test and can be conducted for a 4-day acute test or 7-day chronic test. A measurable reduction or inhibition in bioluminescence is an adverse effect. The endpoint used to measure this light reduction is the IC50 (a 50% reduction in light output when compared to control cells).

A series of tests were conducted to measure the acute effects of marine sediment pore waters with QwikLite. Sediment samples were collected from San Diego Bay and San Francisco Bay, where extraction of pore water samples by centrifugation were analyzed for toxicity. Much of the demonstrated toxicity at the sample sites is believed due to ambient levels of ammonia present in the pore waters and not due to metal or PAH contamination. QwikLite (QwikSed) IC50s were only observed in pore waters exceeding 1.6-ppm total ammonia. Independent toxicity tests conducted at the same sites with the sea urchin embryo development test *(Arabacia)* showed a high correlation (EC50s) with the QwikLite IC50s demonstrating that both tests are sensitive to ammonia. These observations are critical in understanding the role of confounding influences on evaluating sediment toxicity.

## Introduction

Many species of dinoflagellates produce bioluminescence as part of their daily physiological process. A bioassay has been developed in our laboratory that makes use of this ability to produce light for assessment of toxic effects when the dinoflagellates are exposed to many chemicals, either individually or in compounds, effluents, and antifoulant coatings.

Successful assays using the QwikLite bioassay system have provided data on acute responses as well as chronic effects (3 hours to 11 days) on the bioluminescent dinoflagellate *Gonyaulax polyedra*.

## Instrumentation

The QwikLite bioassay system (figure 1) consists of a horizontally-mounted 2-inch-diameter 8575 photomultiplier tube (PMT) attached to the QwikLite test chamber that is connected to the controller box via a combined power and signal cable. The top of the test chamber is removable and houses a small adjustable speed motor that drives a plastic stirrer (figure 2). The controller box displays stirring motor and PMT voltages, PMT counts, time countdown, and cycle status. It has manual and automatic switches for system operation, time settings, start, stop, and reset buttons. Neutral density optical filters (ND-1, ND-2, ND-3) can be easily changed to prevent PMT saturation.

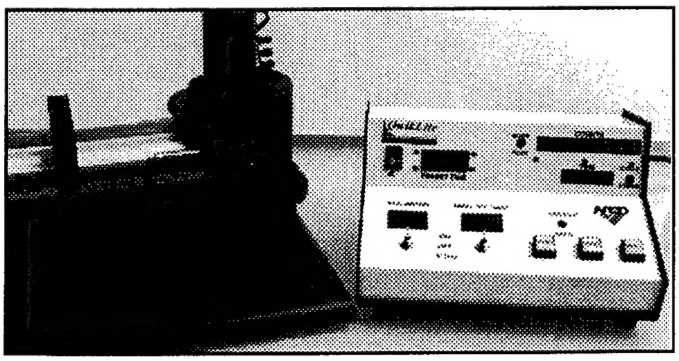


Figure 1. QwikLite bioassay system. On the left, the PMT is bolted to the test chamber. The controller box, on the right, provides power to the PMT, stir motor, and displays PMT counts.

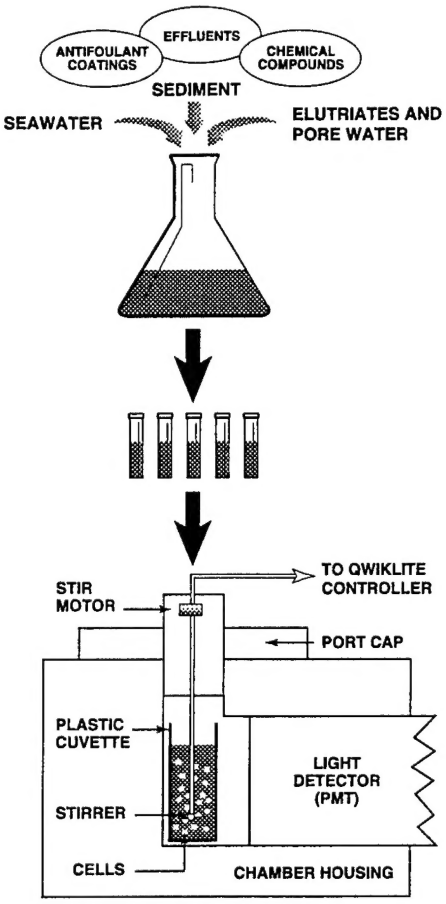



Figure 2. Simplified schematic of the QwikLite test chamber showing a cuvette containing bioluminescent dinoflagellates seated in front of the light detector.

The QwikLite bioassay system was used in the automatic mode for light output testing. In this setting, the high voltage and the stir motor were automatically engaged after the start button was pushed. Stirring created shear forces within the cuvette, stimulating the dinoflagellates to emit light that was detected by the photomultiplier tube (PMT). During stirring, PMT counts were accumulated and displayed on a timer-counter.

Testing typically took less that 1 hour to run five replicates for each of five pore water concentrations and a control.

## Bioassay Organisms

Dinoflagellates were maintained in sterile enriched seawater medium (ESM) under 40-watt cool-white fluorescent bulbs on a 12:12h (light:dark) cycle at 19-20° C. Cells were cultured in ~600 mL ESM in borosilicate Erlenmeyer flasks at 2000-3000 cells/mL. Bioluminescent dinoflagellates (figure 3) are most stimulatable and produce maximum light during their dark phase, so the day:night cycle was reversed to accommodate daytime testing.

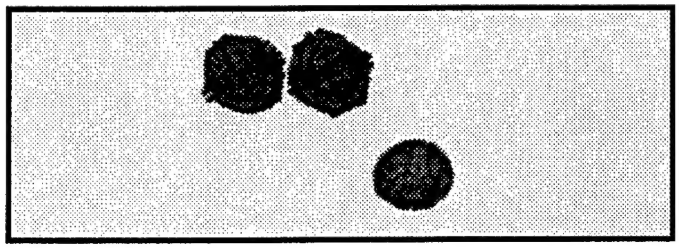


Figure 3. Photomicrograph of the bioluminescent dinoflagellate *Gonyaulax polyedra*. Cell size = 60 µm.

Bioluminescence inhibition is strongly correlated to inhibition of phototaxis behavior and chlorophyll fluorescence. The impact of toxicity on the mobility of dinoflagellates is serious for an organism restricted to the euphotic zone. Inhibition of all three functions suggests significant deleterious changes in the physiology of the dinoflagellates. Data indicate that copper sulfate inhibited bioluminescence, chlorophyll fluorescence, and phototaxis in *G. polyedra* at comparable concentrations within 48 hours of exposure. IC50s of 78 ppb, 70 ppb, and 65 ppb copper sulfate were measured in chlorophyll fluorescence, bioluminescence, and phototaxis, respectively (figure 4).

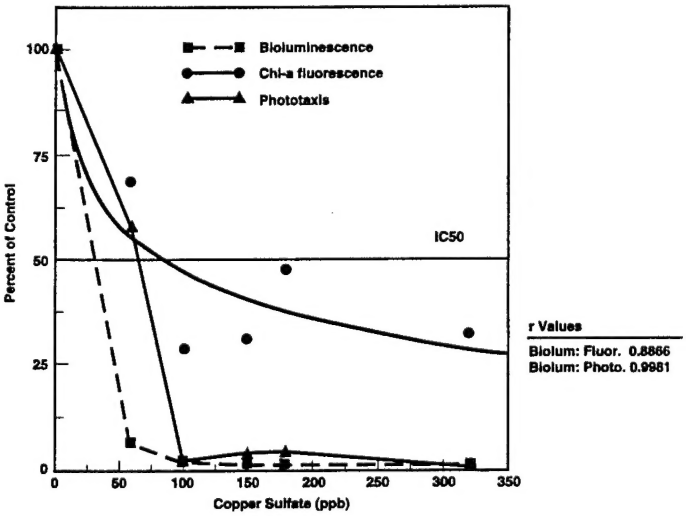



Figure 4. Effect of copper sulfate on bioluminescence, chl-a fluorescence, and phototaxis.

## Methods

In previous work, *G. polyedra* (figure 3) has been observed to be sensitive to numerous metals (table 1, figure 5) and to the reference organic toxicant sodium dodecyl sulfate (SDS) (table 2). *G. polyedra* appears to be more sensitive to some of these metals when compared to the 96-hour mysid shrimp (*Mysidopsis bahia*) static renewal toxicity test (Table 1). *G. polyedra* is also more sensitive to SDS than silverside minnows (*Menidia beryllina*), sheephead minnows (*Cyprinodon variegatus*), the sea urchin (*Arabacia puntulata*), and the mysid shrimp (*Mysidopsis bahia*) (table 2).

Table 1. Results for several bioassays conducted using the QwikLite bioassay system. Note that bioluminescent dinoflagellates were used for both acute and chronic exposures.

| TOXICANT | Duration | $IC_{50}$ | Mysid* $LC_{50}$ |
|---|---|---|---|
| Tributyltin | 196 hrs. | 1.6 ppb | 0.5 ppb† |
| Silver | 96 hrs. | 13 ppb | 249 ppb |
| Copper Sulfate | 96 hrs. | 23 ppb | 120-140 ppb |
| Dibutyltin | 96 hrs. | 34 ppb | — |
| Lead | 96 hrs. | 321 ppb | 3130 ppb |
| Zinc | 96 hrs. | 430 ppb | 499 ppb |
| Chromium | 96 hrs. | 538 ppb | 2030 ppb |
| Cadmium | 96 hrs. | 782 ppb | 110 ppb |

* = Kuhn–Hines et al., 1995 † = Davidson et al., 1986

Table 2. Comparisons of response to reference toxicant sodium dodecyl sulfate (SDS).

| SPECIES | ENDPOINT | $IC_{50}/LC_{50}$ (mg/l) |
|---|---|---|
| *Gonyaulax polyedra* (Dinoflagellate - QwikLite | Bioluminescence | 1.4 |
| *Menidia beryllina* (Silverside minnow) | Larval Survival | 1.8 |
| *Cyprinodon variegatus* (Sheepshead minnow) | Larval Survival | 2.9 |
| *Arabacia punctulata* (Sea Urchin) | Fertilization | 3.2 |
| *Mysidopsis bahia* (Mysid shrimp) | Survival | 9.3 |

(From Johnston, R.J., 1996)

Recent sediment toxicity studies (QwikSed) were conducted to assess the marine sediment collected at San Diego Bay, CA and San Francisco Bay, CA. Sediment samples were collected at Naval Submarine Base (San Diego Bay) and at Naval Air Station Alameda (San Francisco Bay) for analysis and prepared as sediment leachates by mixing sediment with filtered seawater in a 1:4 ratio for 1.5 hours (EPA, 1991). QwikSed testing was also performed on sediment collected from Treasure Island (San Francisco Bay) with pore waters, which were prepared by centrifugation. The salinity of the leachates and pore waters were checked and adjusted with sea salts to a standard salinity of 33‰ prior to testing. Leachates and pore waters were diluted to 6.25% to find the IC50 of each sample. Total ammonia in each sample was measured with the HACH Spectrophotometer or an Orion Ammonia Electrode. Total Polycyclic Aromatic Hydrocarbons (PAHs) in sediment leachates were also measured by UV-Fluorescence Spectroscopy.

Solutions of pore waters or leachates were prepared with an enriched seawater medium (ESM) and dinoflagellates at a concentration of approximately 200 cells/mL. Three mL aliquots from each test concentration (ESM volume, pore water volume, dinoflagellate cell stock volume) were dispensed into five replicates for each test concentration and controls.Cells were cultured directly in disposable optical-grade spectrophotometric plastic cuvettes at 19°C under a light intensity of 4000 lux. Bioluminescence measurements were conducted in consecutive 24- hour increments following test setup. For some of the earlier metals toxicity work, testing was conducted throughout a 96-hr period. Sediment leachate and pore water toxicity testing was conducted with only 24 hours of exposure to the cells.

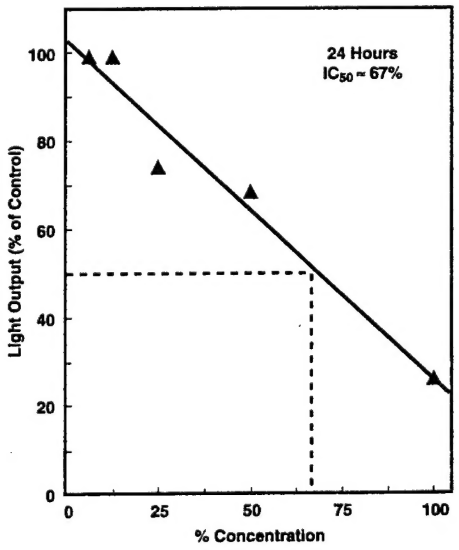



Figure 5. Representative $IC_{50}$ plot showing toxicity of DBT to *G. polyedra* after 24 hours. The DBT was found in a leachate solution from an experimental coating.

At the conclusion of each bioassay, mean light outputs were calculated and graphed as bioluminescence (percent of control) versus concentration of a particular metal or pore water. The IC50 can be calculated using statistics software or graphically plotting concentration of a test material (pore water, metals, etc.) against bioluminescence (figure 5).

## Results

### Use of QwikLite for Sediment Testing (QwikSed) at San Diego Bay and San Francisco Bay

Results from QwikSed tests at San Diego Bay (Naval Submarine Station) and at San Francisco Bay (Naval Air Station Alameda and Treasure Island) showed effects of total ammonia and/or polycyclic aromatic hydrocarbons (PAHs) on assessing sediment toxicity. At Treasure Island in San Francisco Bay, IC50s correlated with total ammonia measurements in pore waters (figure 6). Much of the demonstrated toxicity at the 14 sampled sites is believed to be due to ambient levels of ammonia present in the pore waters. QwikSed IC50s were only observed in pore waters exceeding 1.6-ppm total ammonia. Independent toxicity tests conducted at the same sites with the sea urchin embryo development test *(Arabacia)* showed high correlation with the QwikSed IC50s (figure 7) demonstrating that both toxicity tests are sensitive to ammonia.

Another factor that needs to be addressed in sediment toxicity is the role of total PAHs on QwikSed tests. In the sediment leachates tested from Alameda in San Francisco Bay, QwikSed IC50s correlated with PAHs (figure 8). Low measurements of total PAHs reflected low levels of toxicity. Toxicity (IC50s 15-30%) was detected at sites with high measurements of total PAH (100-135 ppm).

The influence of total ammonia and total PAH on sediment toxicity is also evident in the study at San Diego Naval Submarine Station (figure 9). The majority of the samples showed low levels of ammonia with variable

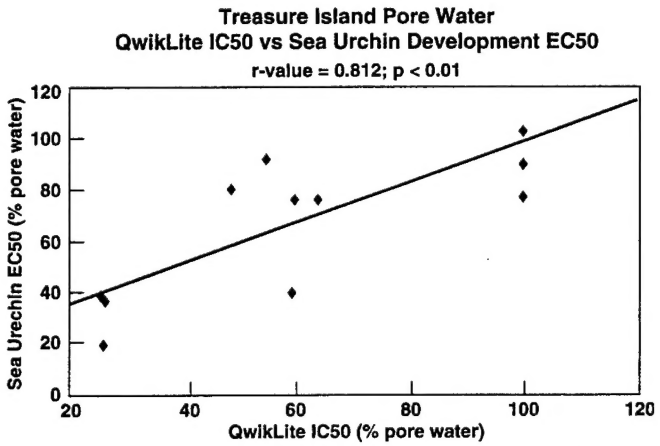



Figure 7. Relationship of QwikSed IC50 vs. sea urchin embryo development EC50.

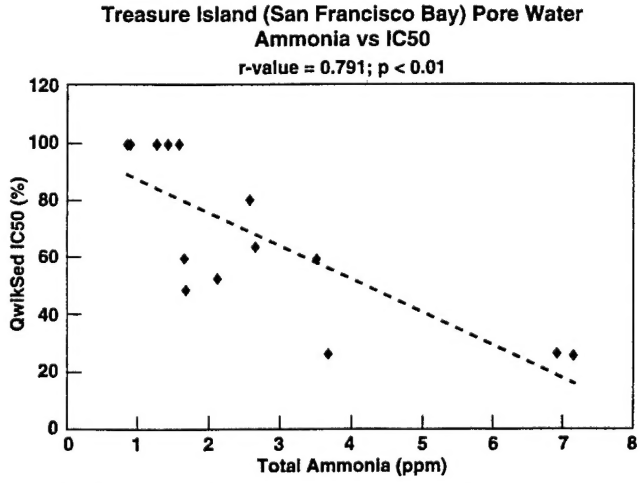



Figure 6. Relationship of total ammonia and toxicity in QwikSed.

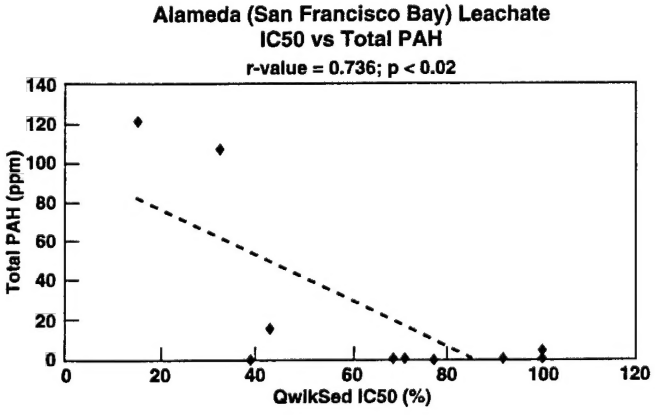



Figure 8. Relationship of QwikSed IC50 vs total PAH.

amounts of PAH. However, ammonia and PAH levels were influential to QwikSed tests at 2 sites; ammonia levels (2-12 ppm) and PAH levels (45-50 ppm) corresponded with IC50s (6-10%). This toxicity is believed to be a result of both ammonia and PAH contamination. These observations are critical in understanding their role of confounding influences on sediment toxicity. It is important to determine if sediment toxicity is a result of one or both of these influences.

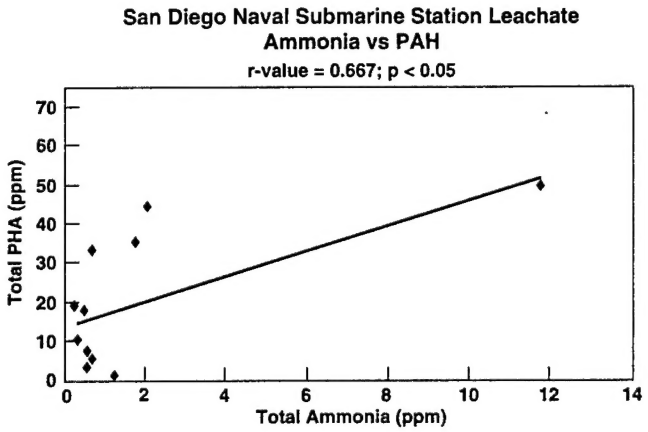



Figure 9. Relationship of total ammonia and total PAH in sediment leachate.

## Conclusions

■ QwikLite bioassays can evaluate both acute and sublethal chronic effects from exposure to a variety of toxicants.

■ QwikLite and QwikSed have a quick and easy initial setup and testing can be accomplished in less than 1 hour per day.

■ QwikLite and QwikSed toxicity tests are as sensitive or more sensitive than mysid shrimp, minnows, chain diatoms, and sea urchins.

■ QwikLite toxicity tests can monitor both acute and chronic effects from exposure to a variety of toxicants.

■ More work is currently being planned to investigate "false positives" from contaminated marine sediments. Ammonia has demonstrated confounding influence on conventional and QwikSed toxicity test results

## References


**Davidson, B. M., A. O. Valkirs and P. F. Seligman.**

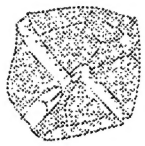
1986. Acute and chronic effects of tributyltin on the mysid *Acanthomysis sculpta* (Crustacea, Mysidacea). In: Proceedings of Oceans '86 Organotin Symposium, Vol. 4. 25-27 September 1986, Washington, DC, pp. 1219-1225.

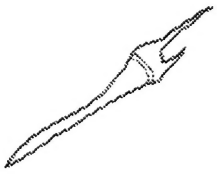
**Johnston, R. J.**

1996. University of Rhode Island (Pers.comm.)

**Kuhn-Hines, A., Munns, Jr., W. R., Lussier, S. M., Champlin, A., and Poucher, S.**

1995. Evaluation of the ecological relevance of mysid toxicity tests using population model techniques, 2nd World Congress, SETAC, Vancouver, British Columbia, Canada.





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# REPORT DOCUMENTATION PAGE

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| 04-1998 | Special Document | |

**4. TITLE AND SUBTITLE**

Use of a Rapid Bioluminescent Bioassay (QwikLite) Using Oceanic Dinoflagellates to Assess Toxicity in Sediments

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHORS**

Lapota, D., SSC San Diego
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**5d. PROJECT NUMBER**

ME65

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

SSC San Diego
53560 Hull Street
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Computer Sciences Corporation
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San Diego, CA 92110-5164

**8. PERFORMING ORGANIZATION REPORT NUMBER**

SD 087

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION/AVAILABILITY STATEMENT**

Approved for public release; distribution is unlimited.

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

This Special Document describes the rapid bioassay system (QwikLite) developed by the Navy.

**15. SUBJECT TERMS**

bioluminescent dinoflagellate
rapid bioassay system (QwikLite)
sediments
toxicity

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | David Lapota, D362 |
| U | U | U | UU | 7 | 19B. TELEPHONE NUMBER *(Include area code)* (619) 553-2798 |